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Citation for published version:

Yu, X, Wang, Y, Kristic, J, Dong, J, Chu, X, Ge, S, Wang, H, Fang, H, Gao, Q, Liu, D, Zhao, Z, Peng, H, Bakovic, MP, Wu, L, Song, M, Rudan, I, Campbell, H, Lauc, G & Wang, W 2016, 'Profiling IgG N-glycans as potential biomarker of chronological and biological ages: A community-based study in a Han Chinese population', *Medicine*, vol. 95, no. 28, e4112. <https://doi.org/10.1097/MD.00000000000004112>

Digital Object Identifier (DOI):

[10.1097/MD.00000000000004112](https://doi.org/10.1097/MD.00000000000004112)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Medicine

Publisher Rights Statement:

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Profiling IgG N-glycans as potential biomarker of chronological and biological ages

A community-based study in a Han Chinese population

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Abstract

As an important post-translation modifying process, glycosylation significantly affects the structure and function of immunoglobulin G (IgG) molecules and is essential in many steps of the inflammatory cascade. Studies have demonstrated the potential of using glycosylation features of IgG as a component of predictive biomarkers for chronological age in several European populations, whereas no study has been reported in Chinese. Herein, we report various patterns of changes in IgG glycosylation associated with age by analyzing IgG glycosylation in 701 community-based Han Chinese (244 males, 457 females; 23–68 years old). Eleven IgG glycans, including FA2B, A2G1, FA2[6]G1, FA2[3]G1, FA2[6]BG1, FA2[3]BG1, A2G2, A2BG2, FA2G2, FA2G2S1, and FA2G2S2, change considerably with age and specific combinations of these glycan features can explain 23.3% to 45.4% of the variance in chronological age in this population. This indicates that these combinations of glycan features provide more predictive information than other single markers of biological age such as telomere length. In addition, the clinical traits such as fasting plasma glucose and aspartate aminotransferase associated with biological age are strongly correlated with the combined glycan features. We conclude that IgG glycosylation appears to correlate with both chronological and biological ages, and thus its possible role in the aging process merits further study.

Abbreviations: ADCC = antibody-dependent cellular cytotoxicity, ALT = alanine aminotransferase, AST = aspartate aminotransferase, BMI = body mass index, Cr = creatinine, DBP = diastolic blood pressure, Fc = crystallizable fragment, FPG = fasting plasma glucose, GlcNAc = N-acetylglucosamine, GP = glycan peak, HDL = high-density lipoprotein, Ig = immunoglobulin, IgG = immunoglobulin G, LDL = low-density lipoprotein, MCV = mean corpuscular volume, SBP = systolic blood pressure, TC = total cholesterol, UA = uric acid, UPLC = ultra-performance liquid chromatography, WBC = white blood cell count.

Keywords: biological age, biomarker, chronological age, immunoglobulin G, N-glycan

Editor: Bernhard Schaller.

Funding: This work was supported by grants from Australian National Health & Medical Research Council and National Natural Science Foundation of China (NHMRC-APP1112767-NSFC 81561128020), and National Natural Science Foundation of China (81273170, 81370083, 81001281, 81373099, 81573215), the National "12th Five-Year" Plan for Science and Technology Support, China (2012BAI37B03), Connecting Australia–Europe Science and Innovation Excellence (CAESIE—Priming). XY and SG were supported by the Chinese Scholarship Council (CSC-2015). MS was supported by the Importation and Development of High-Calibre Talents Project of Beijing Municipal Institutions (CIT&TCD201404185), and YW was supported by Beijing Higher Education Young Elite Teacher Project (YETP1671) and Beijing Nova Program (Z141107001814058).

Authors' contributions: This study was conceived and designed by WW, GL, and IR. Blood samples and clinical data were collected by XY, YW, JD, XC, SG, HW, HF, QG, DL, ZZ, HP, and LW. Data were analyzed by XY, SG, JK, and MPB. Article was written by XY, WW, SG, GL, HC, JK, MPB, MS, and IR. All authors meet ICMJE criteria for authorship.

The authors have no conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

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Medicine (2016) 95:28(e4112)

Received: 22 March 2016 / Received in final form: 3 June 2016 / Accepted: 10 June 2016

<http://dx.doi.org/10.1097/MD.00000000000004112>

1. Introduction

Aging is a progressive process of loss of physiological integrity, including molecular, cellular, and organ impairment, and finally increased chronic inflammation and vulnerability to disease and death.^[1] Decades of research demonstrate that more active and healthier lifestyle such as continued physical activity and smoking cessation may delay the aging process.^[2,3] With these observations, research in screening molecular markers of age to predict or monitor the age-associated physiological decline and disease has experienced an unprecedented advance over recent years.^[1]

Perturbed proteostasis such as secretion of unfolded, misfolded, or aggregated proteins and incorrect degradation of proteins has been proved to be associated with aging and age-related pathologies.^[4–7] The polypeptide sequence of a protein will not change with age because it is defined by the sequence of nucleotides in the relevant genes. However, as an intricate post-translation modifying process, over half of protein and almost all membrane and secreted proteins are glycosylated during the formation process.^[8] Structural changes of the attached glycans are of great physiological significance and many pathological conditions are associated with various types of glycan changes.^[9,10] Diverse plasma glycans were shown to be closely related to aging over 25 years ago^[11] and the associations have also been replicated in recent studies in European^[12–14] and Chinese population.^[15]

Immunoglobulins (Igs) are a group of proteins with antibody activity (immunoglobulin G [IgG], IgM, IgA, IgE, and IgD) accounting for approximately 20% of plasma proteins, and among them IgG is the most abundant (>80%).^[16] IgG is considered as a perfect model glycoprotein because of its well-defined glycan structures and functions.^[10] It consists of 2 identical light chains and heavy chains, which are joined by covalent bonds to form a “Y-shaped” structure.^[17] The 2 domains of IgG that are responsible for their *in vivo* properties are the antigen-binding fragment and the crystallizable fragment (Fc).^[18] A single, N-linked glycosylation site exists at the amino acid 297 in the heavy chain of all IgG subclasses.^[19] The fine changes in attached glycan composition can dramatically change the conformation of the Fc domain with significant consequences for effector functions of IgG.^[10] The addition of terminal sialic acid to this glycan reduces Fcγ receptors (FcγRs) binding and converts IgG antibodies from pro-inflammatory to anti-inflammatory mediators through the lectin receptor SIGN-R1 or DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin), which upregulates surface expression of the inhibitory Fc receptor, FcγRIIb, on inflammatory cells, thereby attenuating autoantibody-initiated inflammation.^[20] Another important role that glycosylation plays in IgG is modulation of antibody-dependent cellular cytotoxicity (ADCC): the addition of fucose residue in the core of the glycan (core fucose) modifies the conformation of the Fc domain in a way to significantly reduce its affinity for FcγRIIIa; therefore, core-fucosylated IgGs are less efficient in activating ADCC.^[21] Most of IgGs (>95%) are core fucosylated, which can prevent them from wrong elicitation of ADCC.^[22] Malfunction of IgG glycosylation appears to be associated with the incidence and development of many inflammatory and autoimmune diseases, such as rheumatoid arthritis,^[23,24] systemic lupus erythematosus,^[25,26] psoriatic arthritis,^[27] Alzheimer disease, and progressive mild cognitive impairment.^[28] Meanwhile, the level of galactosylation in female Japanese was suggested to decrease with aging.^[29]

Considering the crucial role of IgG glycans in inflammatory cascade, and because aging is an inflammation-related progress,

researchers have been investigating the glycosylation changes of IgG or total plasma proteins with age in several European populations.^[30–33] Their observed results from the total plasma glycome cannot eliminate the effects from various concentrations of other plasma proteins. In this study, we focused on the glycosylation of IgG and analyzed 701 individuals from a community-based Han Chinese population to show the changes in IgG glycosylation with age.

2. Materials and methods

2.1. Study design and participant recruitment

This study has been approved by the Ethics Committee of the Capital Medical University, Beijing, China. The ethics approval was given in compliance with the Declaration of Helsinki (World Medical Association, 2000). Each participant included in this study signed informed consent.

An observational cross-sectional study was conducted during 2012, and a total of 701 (23–68 years old) participants of Chinese Han ancestry were recruited from a community-based survey in Beijing. The volunteer participants who met the following inclusion criteria were recruited: no physical and mental abnormalities history registered in their medical records and no medication history during the past 2 weeks. Individuals diagnosed with serious diseases involving the cardiovascular and cerebrovascular system, respiratory system, genitourinary system, digestive system, and hematic system were excluded.

2.2. Collection of clinical traits

Each participant was required to complete a thorough health examination, which consisted of >30 test items, including anthropometric measurements, and physical and chemical properties of blood. For the anthropometric traits, physical examinations and interviews were carried out by trained nurses and physicians. For the blood traits (hematology and biochemical traits), fasting blood samples were collected in the morning after an overnight fasting by venipuncture for standard blood chemistry and hematology assays (Hitachi Automatic Analyzer, Model-7600, Tokyo, Japan), which were conducted at the Medical Laboratory of Xuanwu Hospital, Beijing. After excluding results of qualitative tests and results with missing value, the following 38 clinical traits were assessed in the current study:

- Results of anthropometric tests:* Height (in centimeters) and weight (in kilograms) were measured after participants removed their shoes and hats, any heavy objects from their pockets, and any heavy or bulky outer sweaters or jackets. Body mass index (BMI, kg/m²) was calculated as weight in kilograms divided by height in meters squared. Waist circumference (cm) measurement was taken by wrapping a measuring tape around the natural waist at the navel and hip circumference (cm) measurement was taken over the fullest part of the hips horizontally. Systolic blood pressure (SBP, mmHg) and diastolic blood pressure (DBP, mmHg) were measured twice on the right arm by trained nurses using a standard mercury sphygmomanometer with the participants resting at least 5 min in a sitting position.
- Results of biochemical assays:* These included fasting plasma glucose (FPG, mmol/L), total cholesterol (TC, mmol/L), triglyceride (mmol/L), low-density lipoprotein (LDL, mmol/L), high-density lipoprotein (HDL, mmol/L), alanine aminotransferase (ALT, IU/L), aspartate aminotransferase (AST,

IU/L), creatinine (Cr, $\mu\text{mol/L}$), uric acid (UA, $\mu\text{mol/L}$), and urea (mmol/L).

- (c) **Results of hematology assays:** These comprised white blood cell count (WBC, $10^9/\text{L}$), red blood cell count ($10^{12}/\text{L}$), hemoglobin concentration (g/L), hematocrit (%), blood platelet count ($10^9/\text{L}$), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (pg), mean corpuscular hemoglobin concentration (g/L), neutrophil count ($10^9/\text{L}$), lymphocyte count ($10^9/\text{L}$), eosinophil count ($10^9/\text{L}$), basophil count ($10^9/\text{L}$), monocyte ($10^9/\text{L}$), eosinophil ratio (%), basophil ratio (%), mean platelet volume (fL), platelet hematocrit (%), neutrophil percentage, lymphocyte percentage, monocyte percentage, red cell distribution width (%), and platelet distribution width (%).

2.3. IgG Glycosylation Analysis

2.3.1. Plasma sample preparation. Fasting blood samples were collected from patients in the morning using ethylenediaminetetraacetic acid Vacutainer tube by the trained nurses. The tubes were placed in a cool box with ice blocks and brought to lab within 6 hours. Then the plasma samples were separated after centrifugation at 2000 rpm for 15 minutes and immediately stored at -80°C for glycosylation analysis.

2.3.2. Isolation of IgG from human plasma. The IgG was isolated using protein G monolithic plates as described previously.^[34] Briefly, after washing and equilibrating protein G monolithic plates, 90 μL of plasma was diluted 10 \times with binding buffer (1 \times phosphate-buffered saline, pH 7.4), applied to the protein G plate, and instantly washed. IgGs were eluted with 1 mL of 0.1 M formic acid and immediately neutralized with 1 M ammonium bicarbonate.^[34]

2.3.3. N-Glycan release and labeling. N-Glycan release and labeling of IgG were performed as reported by Menni et al.^[35] Briefly, isolated IgG samples were dried and denatured with addition of 20 μL 2% sodium dodecyl sulfonate (w/v). After incubation at 60°C for 10 minutes, 10 μL of 4% IGEPAL CA-630 and 0.5 mU of PNGase F in 10 μL 5 \times phosphate-buffered saline were added to the samples. The samples were then incubated overnight at 37°C for N-glycan release. The released N-glycans were labeled with 2-aminobenzamide, a fluorescent dye used to make glycans visible by ultra-performance liquid chromatography (UPLC), by multistage mixing with 2-aminobenzamide, dimethylsulfoxide, glacial acetic acid, and 2-picoline borane. Labeled glycans were cleaned and eluted using hydrophilic interaction liquid chromatography–solid phase extraction, and the combined elutes were either analyzed immediately by UPLC or stored at -20°C until usage.

2.3.4. Analysis of IgG N-glycans. IgG N-glycans were separated by hydrophilic interaction liquid chromatography on a Waters ACQUITY UPLC instrument (Waters Corporation, Milford, MA) into 24 glycan chromatographic peaks (GP1–24) and quantified as relative contributions of individual peak to the total IgG N-glycome. Structures of glycans in each peak were determined by mass spectrometry and represented by alphanumeric characters (see Table 1, Supplemental Content, <http://links.lww.com/MD/B95>, which illustrates the glycan structures in the IgG glycome). In short, 2 N-acetylglucosamines (GlcNAcs) and 3 mannose residues are combined to form the core sugar sequence for each IgG N-glycan. Based on that core structure, F represents a core fucose α -1 to -6 linked to the inner GlcNAcs; Ax, number

(x) of GlcNAc (antenna) linked to the inner trimannose residues; Gx, number (x) of galactose on antenna-linked GlcNAc; Sx, number (x) of sialic acid residue on galactose; Mx, number (x) of mannose linked to core GlcNAcs; and B, bisecting GlcNAc linked to core mannose.^[34] The minor glycan peak (GP), GP3, was excluded from all the calculations because in some samples it coeluted with a contaminant that significantly affected its value, and GP20 was also eliminated as its glycan structure has not been determined.

2.4. Statistical analysis

2.4.1. Descriptive analysis of glycan structures. Among the total 24 GPs, GP3 and GP20 were excluded from all of the following statistical analyses due to the reason mentioned previously. All analyses were performed with R programming language^[36] (R Core Team, 2013). All reported *P* values were 2-sided, and $P < 0.05$ was considered statistically significant. Normality distributions of all variables were tested by Shapiro–Wilk test (“fBasics” package) (see Tables 2 and 3, Supplemental Content, <http://links.lww.com/MD/B95>, which illustrate the normality tests of IgG N-glycans before and after log transformation). A log transformation was performed on the 22 glycan variables to obtain normally distributed variables. For most of the variables that were not normally distributed, median together with interquartile range was used in descriptive statistics (see Table 4, Supplemental Content, <http://links.lww.com/MD/B95>, which illustrates the description of IgG N-glycans).

Correlation coefficient matrices of IgG N-glycan structures, age, and clinical traits in both sexes were assessed independently by Spearman rank correlation method (“psych” package). Sex differences in IgG N-glycans and clinical traits were tested by *Mann–Whitney U* tests or 2-sample *t* test according to the results of normality tests of the data.

2.4.2. Prediction of chronological age from glycan structures. Based on the assumption of association between glycans and age, and considering the significant difference of glycan structures among sex, we decided to build 3 predictive models of chronological age using the glycan structures with the pooled (males and females), male, and female samples, respectively, that is, 3 models: GlyAge-Pooled, GlyAge-Male, and GlyAge-Female models. Glycan structures that were statistically related with chronological age were used as potential independent variables for the 3 predictive models. The best combination of glycan structures in the final models was determined using all-subsets regression (“leaps” package). Considering the loss of information in nonparametric test, and because not all variables could be successfully transformed to normality, the predictive models of chronological age were built using binominal regression (“stats” package) and permutation test approach (“lmPerm” package). Binominal regression was first applied to estimate the model coefficient, and the best model was determined for each sex according to the Akaike information criterion. The maximal age predictive model included linear and quadratic terms for each of the age-related glycan structures. Finally, the best models based on pooled, male, and female samples were determined separately according to the Akaike information criterion (“aod” package)^[37] and the values of adjusted R-squared and then named as GlyAge-Pooled, GlyAge-Male, and GlyAge-Female models. The GlyAge-Pooled, GlyAge-Male, and GlyAge-Female indexes that integrated different GPs were obtained from each established model.

2.4.3. Association analysis of GlyAge-Pooled, GlyAge-Male, and GlyAge-Female indexes with clinical traits. To identify the clinical (anthropometric, hematological, and biochemical) traits that might be accountable for the differences between predicted age and chronological age, we performed association analysis with the 38 clinical traits in pooled, male, and female samples independently. In pooled samples, we defined 2 equations for each trait:

Predicted age ~ age + sex

Predicted age ~ age + sex + trait

Analysis of variance test ("stats" package) was performed on those equations to identify the traits that can significantly reduce the residual sum of squares of the equations. In male and female samples, we determined the clinical traits that may be responsible for the differences between predicted and chronological ages in a similar way, just without the sex variable.

2.4.4. Prediction of chronological age with glycan structures and clinical traits. The predictive models of chronological age that combined clinical traits and glycan structures were built in the same way as GlyAge-Pooled, GlyAge-Male, and GlyAge-Female models and named as GlyCliAge-Pooled, GlyCliAge-Male, and GlyCliAge-Female accordingly. For the 3 combined models, the clinical traits that were tested to be associated with chronological age and the GPs involved in the previous 3 indexes were included in the maximum model. Additionally, the performances of the 3 combined models were tested on pooled, male, and female samples.

3. Results

3.1. Description of clinical traits

In total, 38 clinical traits (6 anthropometric, 10 biochemical, and 22 hematology traits) of all participants were described and compared in different sexes (Table 1). Most of the traits (30 out of 38) were significantly different between males and females ($P < 0.05$). In terms of anthropometric traits, the levels of BMI, SBP, DBP, and waist circumference in males were significantly higher than those in females. The same situation also occurred in levels of 5 biochemical traits including FPG, triglyceride, ALT, AST, and Cr, whereas higher level of HDL was found in females. For hematology traits, the values of the inflammation-related parameters (WBC, neutrophil count, lymphocyte count, eosinophil count, basophil count, monocyte, neutrophil percentage, and monocyte percentage) were higher in males. To some extent, all the aforementioned traits are well known to be correlated with inflammatory cascade, inflammation-linked diseases, or aging process.^[1,38]

3.2. IgG N-glycan structures of participants

IgG N-glycan analysis was performed on 701 (244 males, 457 females) Chinese Han participants. Monogalactosylated glycan (FA2[6]G1) had the highest concentrations in pooled and male samples (median: 18.58% and 18.69%, respectively), whereas the digalactosylated glycan (FA2G2) accounts for the largest proportion of the glycan structures in females (median: 18.91%). Among the total 22 GPs, the values of 10 GPs (FA1, A2, FA2, M5, FA2B, A2G1, FA2[6]BG1, FA2[3]BG1, FA2BG2S1, and FA2BG2S2) were significantly higher in males, whereas another 4 GPs (A2G2, FA2G2, FA2BG2, and FA2G2S1) were observed to

Table 1

Difference of clinical traits between males and females*.

Parameters	Pooled	Male	Female	P value
Number, %	701 (100)	244 (34.8)	457 (65.2)	
Age, y	48 (10)	48 (8)	47 (6)	0.050
Anthropometric traits				
BMI, kg/m ²	24.4 (4.1)	25.7 (4.0)	23.8 (3.7)	0.000 [†]
SBP, mmHg	120 (16)	120 (20)	118 (16)	0.000 [†]
DBP, mmHg	80 (16)	80 (10)	79 (10)	0.000 [†]
WC, cm	79 (13)	85 (10)	76 (11)	0.000 [†]
HC, cm	95 (7)	96 (5)	95 (9)	0.654
Heart rate, beats/min	76 (10)	76 (12)	76 (10)	0.340
Biochemical traits				
FPG, mmol/L	5.21 (0.77)	5.45 (0.98)	5.08 (0.69)	0.000 [†]
TC, mmol/L	5.07 (1.23)	5.05 (1.29)	5.09 (1.20)	0.878
TG, mmol/L	1.15 (0.95)	1.58 (1.45)	1.02 (0.75)	0.000 [†]
LDL, mmol/L	2.77 (0.93)	2.86 (1.01)	2.72 (0.88)	0.148
HDL, mmol/L	1.6 (0.43)	1.43 (0.37)	1.70 (0.41)	0.000 [†]
ALT, IU/L	17 (11)	22 (14)	15 (8)	0.000 [†]
AST, IU/L	19 (7)	21 (8)	18 (5)	0.000 [†]
Cr, μ mol/L	54 (17)	69 (13)	50 (8)	0.000 [†]
Urea, mmol/L	5.12 (1.64)	5.60 (1.67)	4.90 (1.50)	0.000 [†]
UA, μ mol/L	248 (112)	326 (85)	215 (72)	0.000 [†]
Hematology traits				
WBC, 10 ⁹ /L	5.99 (1.96)	6.43 (2.33)	5.73 (1.89)	0.000 [†]
RBC, 10 ¹² /L	4.57 (0.55)	4.93 (0.42)	4.41 (0.41)	0.000 [†]
Hb, g/L	138 (21)	154 (13)	132 (13)	0.000 [†]
HCT, %	40.70 (5.10)	44.55 (3.18)	39.30 (3.30)	0.000 [†]
PLT, 10 ⁹ /L	240 (70)	221 (60)	252 (72)	0.000 [†]
MCV, fL	89.90 (5.20)	90.35 (5.28)	89.70 (5.20)	0.000 [†]
MCH, pg	30.7 (1.9)	31.4 (1.5)	30.2 (2)	0.000 [†]
MCHC, g/L	340 (12)	346 (10)	336 (11)	0.000 [†]
NEUT, 10 ⁹ /L	3.42 (1.47)	3.56 (1.73)	3.35 (1.46)	0.000 [†]
LYMPH, 10 ⁹ /L	1.97 (0.75)	2.195 (0.82)	1.91 (0.70)	0.000 [†]
EOS, 10 ⁹ /L	0.10 (0.10)	0.14 (0.14)	0.09 (0.08)	0.000 [†]
BASO, 10 ⁹ /L	0.02 (0.01)	0.03 (0.02)	0.02 (0.02)	0.000 [†]
MONO, 10 ⁹ /L	0.35 (0.15)	0.41 (0.16)	0.32 (0.13)	0.000 [†]
EOSR, %	1.70 (1.60)	2.20 (1.88)	1.60 (1.50)	0.000 [†]
BASOR, %	0.4 (0.4)	0.4 (0.3)	0.4 (0.4)	0.318
RDW, %	12.7 (0.8)	12.6 (0.7)	12.7 (0.9)	0.000 [†]
PDW, %	12.30 (2.40)	12.45 (2.70)	12.30 (2.30)	0.129
MPV, fL	10.80 (1.05)	10.80 (1.18)	10.80 (1.00)	0.558
PCT, %	0.26 (0.08)	0.24 (0.06)	0.27 (0.08)	0.000 [†]
NEUT%	57.50 (10.85)	56.00 (9.93)	58.50 (11.55)	0.007 [†]
LYMPH%	33.60 (10.15)	34.10 (10.05)	33.40 (9.90)	0.639
MONO%	5.70 (1.90)	6.30 (1.78)	5.60 (1.75)	0.000 [†]

ALT = alanine aminotransferase, AST = aspartate aminotransferase, BASO = basophil count, BASOR = basophil ratio, BMI = body mass index, Cr = creatinine, DBP = diastolic blood pressure, EOS = eosinophil count, EOSR = eosinophil ratio, FPG = fasting plasma glucose, Hb = hemoglobin, HC = hip circumference, HCT = hematocrit, HDL = high-density lipoprotein, LDL = low-density lipoprotein, LYMPH = lymphocyte count, LYMPH% = lymphocyte percentage, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, MCV = mean corpuscular volume, MONO = monocyte, MONO% = monocyte percentage, MPV = mean platelet volume, NEUT = neutrophil count, NEUT% = neutrophil percentage, PCT = platelet hematocrit, PDW = platelet distribution width, PLT = blood platelet count, RBC = red blood cell count, RDW = red cell distribution width, SBP = systolic blood pressure, TC = total cholesterol, TG = triglyceride, UA = uric acid, WBC = white blood cell count, WC = waist circumference.

* Data are presented as median (interquartile range).

[†] $P < 0.05$; difference between male and female is statistically significant.

be of higher values in females ($P < 0.001$), indicating the different level of galactosylation and sialylation between sexes. Compared with females, males had a higher proportion of nongalactosylated (FA1, A2, FA2, M5, FA2B), monogalactosylated (A2G1, FA2[6]BG1, FA2[3]BG1), and disialylated (FA2BG2S2) glycans ($P < 0.001$) (Table 2; see Table 1, Supplemental Content, <http://links>.

Table 2
Descriptive statistics for GPs by sex*.

GP	Pooled, %	Male, %	Female, %	P value (U statistic)
GP1	0.06 (0.05)	0.07 (0.05)	0.06 (0.04)	0.000 [†]
GP2	0.34 (0.25)	0.42 (0.29)	0.30 (0.21)	0.000 [†]
GP4	15.68 (6.15)	16.74 (5.28)	14.57 (6.07)	0.000 [†]
GP5	0.23 (0.09)	0.26 (0.08)	0.22 (0.08)	0.000 [†]
GP6	3.82 (1.59)	4.37 (1.44)	3.53 (1.31)	0.000 [†]
GP7	0.58 (0.34)	0.63 (0.41)	0.56 (0.31)	0.005 [†]
GP8	18.58 (2.25)	18.69 (2.40)	18.53 (2.27)	0.054 [*]
GP9	9.67 (1.73)	9.84 (1.83)	9.58 (1.76)	0.065 [†]
GP10	5.04 (1.24)	5.22 (1.55)	4.89 (1.13)	0.000 [†]
GP11	0.68 (0.17)	0.71 (0.20)	0.66 (0.16)	0.000 [†]
GP12	0.96 (0.61)	0.89 (0.59)	1.00 (0.63)	0.037 [†]
GP13	0.46 (0.16)	0.45 (0.16)	0.47 (0.15)	0.049
GP14	17.95 (5.1)	16.55 (3.59)	18.91 (5.01)	0.000 ^{†,‡}
GP15	1.96 (0.58)	1.90 (0.57)	1.99 (0.58)	0.017 [†]
GP16	3.07 (0.69)	3.09 (0.73)	3.06 (0.69)	0.223 [†]
GP17	0.99 (0.32)	0.98 (0.34)	1.00 (0.32)	0.110
GP18	12.06 (4.04)	11.04 (3.01)	12.72 (4.25)	0.000 [†]
GP19	1.88 (0.44)	1.92 (0.49)	1.86 (0.42)	0.005 [†]
GP21	0.68 (0.21)	0.69 (0.23)	0.67 (0.20)	0.167
GP22	0.10 (0.05)	0.1 (0.05)	0.1 (0.05)	0.172
GP23	1.65 (0.80)	1.55 (0.83)	1.69 (0.78)	0.079
GP24	1.60 (0.59)	1.66 (0.61)	1.56 (0.60)	0.043 [†]

GP = glycan peak, IgG = immunoglobulin G.

* Data are presented as median (interquartile range).

[†] $P < 0.05$; difference between male and female is statistically significant.

[‡] Those variables are normally distributed and the difference between male and female was tested using 2-sample *t* test. GP values were presented as percentages of the total IgG glycome. GP1 to 24 represent GP values 1 to 24.

lww.com/MD/B95, which illustrates the glycan structures in the IgG glycome), that is, the males had a lower level of galactosylation and a higher level of disialylation.

3.3. Relationship between IgG glycosylation and chronological age

Nineteen (GP1, 2, 4–15, 17–19, 21, and 23) out of 22 GPs in the pooled samples were statistically significantly associated with age. Among these 19 GPs, 11 GPs (GP2, 4, 6, 8, 10, 11, 13, 14, 18, 19, and 23) in males and 18 GPs (GP1, 2, 4–15, 17, 18, 21, and 23) in females were found to be related with age. Meanwhile, 10 GPs (GP2, 4, 6, 8, 10, 11, 13, 14, 18, and 23) that represent the structures of A2, FA2, FA2B, FA2[6]G1, FA2[6]BG1, FA2[3]BG1, A2BG2, FA2G2, FA2G2S1, and FA2G2S2, respectively, were constantly correlated with age in all 3 groups (pooled, male, and female samples) and the association was stronger in females ($0.203 < |R| < 0.576$, $P < 0.001$) than in males ($0.135 < |R| < 0.368$, $P < 0.001$).

The top glycan structure correlated with age is FA2B, a nongalactosylated nonsialylated glycan, in both males ($R = 0.368$, $P < 0.001$) and females ($R = 0.576$, $P < 0.001$). Nongalactosylated (A2, FA2, and FA2B) and monogalactosylated (FA2[6]BG1 and FA2[3]BG1) glycans steadily increased with age, whereas digalactosylated glycans (A2BG2, FA2G2, FA2G2S1, and FA2BG2S2) steadily decreased with age in both males and females. The only exception was the monogalactosylated glycan without bisecting GlcNAc (FA2[6]G1), which decreased with age in males ($R = -0.135$, $P = 0.04$) but increased in females ($R = 0.223$, $P < 0.001$) (Table 3). The relationship between glycan levels and age was changed more dramatically in females than in males. Most overlap of the fitted value in different sexes was accorded between 50 and 60 years of age, which was correlated with the time when most of the women had gradually adapted to the menopause (Fig. 1; see Fig. Supplemental Content, <http://links.lww.com/MD/B95>, which illustrates the relationship between age and glycan structures).

Although the association between sialylation and age was more complicated, the biantennary digalactosylated glycans with 1 or 2

Table 3
Associations of immunoglobulin G glycans with age.

GP	Pooled		Male		Female	
	R	P value	R	P value	R	P value
GP1	0.248	0.000	0.108	0.092	0.306	0.000
GP2	0.340	0.000	0.147	0.022	0.415	0.000
GP4	0.419	0.000	0.264	0.000	0.477	0.000
GP5	0.223	0.000	0.075	0.241	0.279	0.000
GP6	0.516	0.000	0.368	0.000	0.576	0.000
GP7	0.095	0.012	0.007	0.916	0.123	0.008
GP8	0.091	0.016	-0.135	0.035	0.223	0.000
GP9	0.108	0.004	0.009	0.884	0.163	0.000
GP10	0.318	0.000	0.263	0.000	0.335	0.000
GP11	0.327	0.000	0.302	0.000	0.313	0.000
GP12	-0.235	0.000	-0.109	0.089	-0.305	0.000
GP13	-0.195	0.000	-0.178	0.005	-0.203	0.000
GP14	-0.504	0.000	-0.367	0.000	-0.536	0.000
GP15	-0.173	0.000	0.041	0.522	-0.288	0.000
GP16	0.062	0.104	0.099	0.124	0.024	0.609
GP17	-0.180	0.000	-0.056	0.383	-0.254	0.000
GP18	-0.443	0.000	-0.250	0.000	-0.512	0.000
GP19	0.074	0.049	0.213	0.001	-0.035	0.455
GP21	-0.081	0.031	-0.065	0.314	-0.107	0.022
GP22	-0.027	0.481	-0.055	0.393	-0.023	0.631
GP23	-0.202	0.000	-0.156	0.015	-0.220	0.000
GP24	0.062	0.099	0.104	0.106	0.018	0.697

GP1 to 24 represent GP values 1 to 24. GP = glycan peak, R = Spearman correlation coefficient.

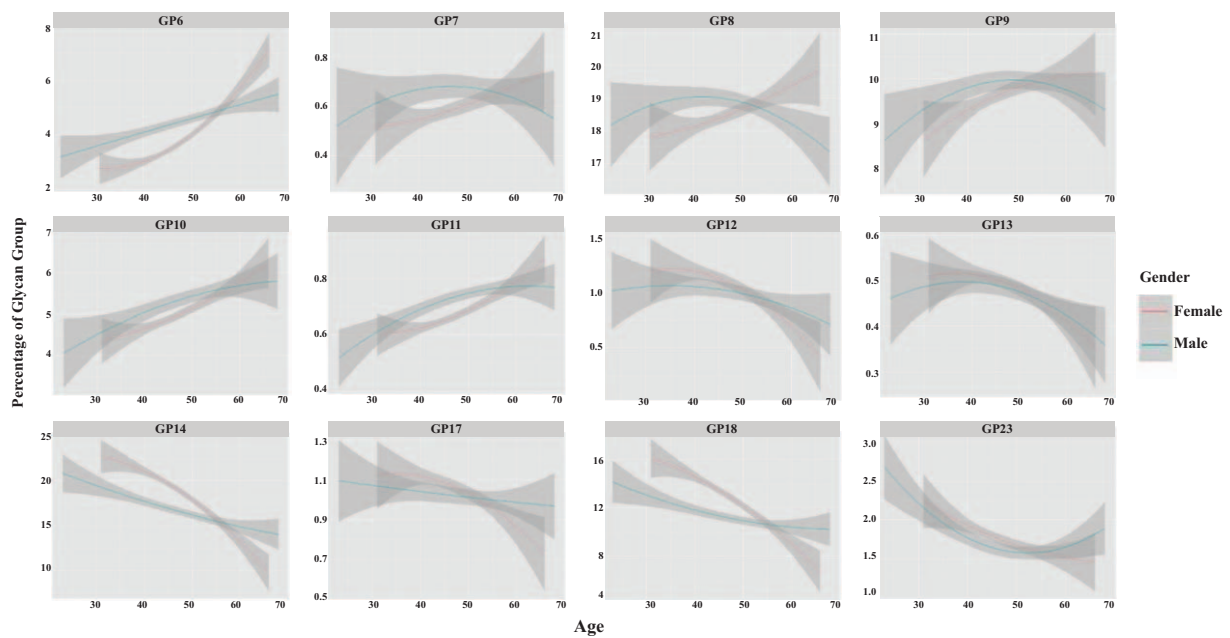


Figure 1. The line graph of relationship between age and selected glycan structures. Plots describe associations between the individual contributions of 12 selected glycan structures to the total IgG glycome and chronological age. Blue and red curves are fitted linear regression models. The shaded region is the 95% confidence interval on the fitted values. GP = glycan peak, IgG = immunoglobulin G.

terminal sialic acid (FA2G2S1, FA2G2S2) significantly decreased with age in both males and females.

3.4. Predictive model of chronological age from IgG N-glycans

As different groups of glycans were related with chronological age in pooled, male, and female samples, 10 age-related GPs were used for the construction of GlyAge-Pooled model, 11 GPs for GlyAge-Male model, and 18 GPs for GlyAge-Female model. The GlyAge indexes in pooled, male, and female samples composed of the following different glycan variables:

GlyAge-Pooled model:

$$\text{Predicted age} = 73.5 - (1.1 \times \text{GP8}^2) + (2.1 \times \text{GP10}^2) - (2.2 \times \text{GP14}^2) - (0.7 \times \text{GP18}^2)$$

GlyAge-Male model:

$$\begin{aligned} \text{Predicted male age} = & 54.5 + (39.5 \times \text{GP6}) - (14.2 \times \text{GP6}^2) - (2.5 \times \text{GP8}^2) + (3.2 \times \text{GP10}^2) \\ & + (2.5 \times \text{GP13}^2) - (2.8 \times \text{GP14}^2) \end{aligned}$$

GlyAge-Female model:

$$\begin{aligned} \text{Predicted female age} = & -1.9 + (6.1 \times \text{GP7}) + (10.7 \times \text{GP9}) + (11.8 \times \text{GP10}) - (9.2 \times \text{GP11}) - (7.94 \times \text{GP12}) \\ & + (5.6 \times \text{GP17}) + (2.8 \times \text{GP6}^2) + (1.9 \times \text{GP23}^2) \end{aligned}$$

The GlyAge-Pooled model contains 2 monogalactosylated glycans (FA2[6]G1 and FA2[6]BG1), 1 digalactosylated glycan (FA2G2), and 1 digalactosylated monosialylated glycan

(GA2G2S1), thus covering nearly all elements of IgG glycosylation. This GlyAge-Pooled model explained 29.4% of variation in chronological age in pooled samples and the correlation of age and predicted age was 0.56 ($P < 0.001$). The predictions based on male and female samples were fluctuating, with correlation between age and predicted age of 0.45 and 0.60 ($P < 0.001$) (Table 5).

In terms of GlyAge-Male model, the proportion of explained variation was 23.2% and the correlation of age and predicted age was 0.50 in male samples. The correlations increased to 0.55 and 0.57 ($P < 0.001$) when the GlyAge-Male model was applied in the pooled and female samples, respectively (Table 5). The nongalactosylated, monogalactosylated, and digalactosylated glycans (FA2B, FA2[6]BG1, A2BG2, FA2G2) were all involved in this model, but the impact of sialylation was not included.

For the GlyAge-Female model, up to 45.4% variations in chronological age could be explained in female samples, with the correlation between age and predicted age of 0.65 ($P < 0.001$). However, the correlation coefficient dropped to 0.58 and 0.40 ($P < 0.001$) when GlyAge-Female model was tested in pooled and male samples, respectively (Table 5). This indicated that the associations between age and glycans vary in sexes and the glycans showed stronger correlations with age in females than in males.

3.5. Relationship between IgG glycosylation and biological age

To determine variables that may be responsible for the remaining variability in the GlyAge-Pooled, GlyAge-Male, and GlyAge-Female indexes, we performed an association analysis among all clinical traits. The traits (10 traits in pooled samples, 6 traits in males, and 5 traits in females) such as DBP, FPG, ALT, AST, WBC, and UA with statistically significant association with

Table 4**Association of GlyAge indexes with clinical traits after adjustment of chronological age.**

Traits	GlyAge-Pool index		GlyAge-Male index		GlyAge-Female index	
	Regression coefficient*	P	Regression coefficient†	P	Regression coefficient‡	P
SBP	0.0072	0.375	0.0349	0.030	−0.0077	0.442
DBP	0.0220	0.051	0.0626	0.005	0.0018	0.894
WC	0.0323	0.022	0.0095	0.727	0.0078	0.654
Heart rate	0.0242	0.038	0.0194	0.407	0.0235	0.099
FPG	0.3687	0.010	0.4863	0.042	0.4854	0.016
TC	0.3619	0.003	0.2480	0.298	0.2934	0.061
TG	0.2375	0.004	0.1811	0.203	0.2085	0.061
LDL	0.3381	0.030	−0.0816	0.783	0.3513	0.081
HDL	0.2020	0.538	1.7450	0.018	0.1241	0.744
ALT	0.0195	0.002	0.0093	0.294	0.0326	0.004
AST	0.0540	0.000	0.0500	0.041	0.0502	0.003
Cr	−0.0167	0.224	−0.0287	0.199	0.0111	0.577
WBC	0.1383	0.044	0.3500	0.003	−0.0688	0.453
Urea	0.1501	0.111	0.0793	0.651	0.3572	0.003
UA	0.0088	0.000	0.0064	0.059	0.0095	0.000

ALT = alanine aminotransferase, AST = aspartate aminotransferase, Cr = creatinine, DBP = diastolic blood pressure, FPG = fasting plasma glucose, HDL = high-density lipoprotein, LDL = low-density lipoprotein, SBP = systolic blood pressure, TC = total cholesterol, TG = triglyceride, UA = uric acid, WBC = white blood cell count, WC = waist circumference.

* The regression coefficient was calculated in pooled samples.

† The regression coefficient was calculated in male samples.

‡ The regression coefficient was calculated in female samples.

GlyAge indexes were also well known to be significantly related with unhealthy lifestyles or inflammation process (Table 4; see Table 5, Supplemental Content, <http://links.lww.com/MD/B95>, which illustrates association of GlyAge index with clinical traits after adjustment of chronological age).

Many clinical traits were significantly associated with both the predicted age (predicted by GlyAge-Pooled, GlyAge-Male, and GlyAge-Female models) and the chronological age (see Table 6, Supplemental Content, <http://links.lww.com/MD/B95>, which illustrates association of chronological and GlyAge with clinical traits in pool, male, and female samples). Hence, we attempted to take the information from both clinical traits and glycan structures to build a combined, comprehensive age predictive model.

3.6. Combined predictive model of chronological age from IgG N-glycans and clinical traits

In pooled samples, inclusion of SBP, LDL, hemoglobin, MCV, urea, and UA into GlyAge-Pooled model further improved the prediction power and cumulatively explained 36.1% variation of age in pooled samples and the correlation between age and predicted age was increased to 0.59 ($P < 0.001$). This combined model was named as GlyCliAge-Pooled model. When the

GlyCliAge-Pooled model was tested in male and female samples, the correlations between age and predicted age were 0.51 and 0.61 ($P < 0.001$), respectively. Similarly in male samples, the proportion of explained variance of age increased to 36.7% with correlation between age and predicted male age of 0.47 when FPG, ALT, urea, and MCV were included into GlyAge-Male model. This GlyCliAge-Male model was also applied in other 2 groups of samples, pooled and male samples, and the associations between age and predicted age were nearly the same (0.45 and 0.41). In females, inclusion of clinical traits (SBP, TC, HDL, and Cr) could improve the predictive ability of glyAge-Female model to 48.2% but the correlation between age and predicted age decreased to 0.29 in males, and 0.46 in pooled samples (Table 5). The combined models based on the pooled, male, and female samples are as follows:

GlyCliAge-Pooled model:

$$\begin{aligned} \text{Predicted age} = & 43.39 + (1.98 \times \text{GP10}^2) \\ & - (2.47 \times \text{GP14}^2) + (0.06 \times \text{SBP}) + (1.34 \\ & \times \text{LDL}) + (0.67 \times \text{urea}) - (0.00007 \times \text{Hb}^2) \\ & + (0.0009 \times \text{MCV}^2) - (0.00002 \times \text{UA}^2) \end{aligned}$$

Table 5**Adjusted R-squared and Spearman correlations of chronological age and age predicted by various models.**

Model	Pooled		Male		Female	
	R ² , %	Correlation	R ² , %	Correlation	R ² , %	Correlation
GlyAge	29.4	0.56	—	0.45	—	0.60
GlyAge-Male	—	0.55	23.2	0.50	—	0.57
GlyAge-Female	—	0.58	—	0.40	45.4	0.65
GlyCliAge	36.1	0.59	—	0.51	—	0.61
GlyCliAge-Male	—	0.45	36.7	0.47	—	0.41
GlyCliAge-Female	—	0.46	—	0.29	48.2	0.67
GlyAge-Euro-male*	—	0.54	—	0.41	—	0.57
GlyAge-Euro-female†	—	0.40	—	0.30	—	0.43

* The age predictive model for males built based on European population.

† The age predictive model for females built based on European population.

GlyCliAge-Male model:

$$\begin{aligned} \text{Predicted male age} = & 0.87 + (1.68 \times \text{GP10}^2) - (2.45 \\ & \times \text{GP14}^2) + (11.66 \times \text{FPG}) - (0.13 \times \text{ALT}) \\ & + (0.96 \times \text{urea}) - (0.76 \times \text{FPG}^2) \\ & + (0.0003 \times \text{ALT}^2) + (0.002 \times \text{MCV}^2) \end{aligned}$$

GlyCliAge-Female model:

$$\begin{aligned} \text{Predicted female age} = & 26.41 + (5.89 \times \text{GP7}) - (6.08 \\ & \times \text{GP12}) + (2.33 \times \text{GP6}^2) - (0.04 \\ & \times \text{SBP}) - (1.13 \times \text{TC}) + (8.62 \\ & \times \text{HDL}) - (2.24 \times \text{HDL}^2) \\ & + (0.0007 \times \text{Cr}^2) \end{aligned}$$

4. Discussion

Many epidemiological studies and experimental observations have demonstrated that changes in IgG Fc glycosylation induce pro-inflammatory or anti-inflammatory actions of Igs,^[10,20,39,40] indicating that IgG Fc glycosylation plays an important role in the process of aging.^[29,41] The reduction of terminal galactose residues was suggested as a mechanism to enhance the pro-inflammatory activity *in vitro*.^[42] Our study was consistent with that hypothesis by observing a lower level of galactosylation in males who had a higher WBC, a classic clinical inflammatory index, than that in females. Furthermore, higher sialylation was found in males, which seemed contradictory with findings of previous studies because those results showed the anti-inflammatory properties of binding sialic acid residue to IgG Fc fragment.^[43] However, the observations in our study may still be understood in the following context. First, the anti-inflammatory properties did not only depend on the amount of sialic acid but also required attachment of the oligosaccharides in an $\alpha 2$, 6 linkage.^[44] The mix of 2 different linkage structures ($\alpha 2$, 6 and $\alpha 2$, 3) in this study may weaken the anti-inflammatory activity. Second, although the WBC in males was higher than that in females, both of the values were still in the normal range and all the participants were recruited from the same community with general homogenous environment background. Thus, more heavily sialylated glycan structures might be needed to balance the pro-inflammatory properties caused by the lower galactosylation in males (Tables 1 and 2). Those results indicated that these glycan structures are associated with an inflammatory condition, which is a significant risk factor in the aging process.

A combined genomics and epigenomics study indicated that the structure of glycan moiety is defined by dynamic interactions between their encoding genes, their protein products, and environmental factors.^[45] For the total plasma N-glycans, only 5% of the variance in glycans can be explained by nongenetic variables, such as age, BMI, and plasma lipid profiles.^[33] The average heritability of 24 IgG N-glycan traits in TwinsUK study was 0.49, indicating that the nongenetic factors contributed a little bit more than half of the variance to the levels of those N-glycan traits.^[35] As we can see in our study, a large part of the nongenetic variability in IgG N-glycan structures was able to be explained by age and clinical traits related to age. Most of IgG glycans (19 out of 22 GPs) were significantly related with age in which the prominent trend we observed is the decrease in IgG galactosylation with age (Table 3). Studies in European population showed a similar glycan profiling change more than 15 years ago, but 1 recent investigation indicated that the

galactosylation is increasing with age in children from 6 to 18 years old.^[46] Because the participants in our study were in the age of 23 to 68, it was possible that the level of galactosylation would increase until adolescence (about 18 years old) and then decrease during the maturing life span. In addition, the association patterns between IgG N-glycan traits and age were more complex in community-based study due to the diversity of the patterns in sexes (Fig. 1; see Fig. Supplemental Content, <http://links.lww.com/MD/B95>, which illustrates the relationship between age and glycan structures). High level of galactosylation in IgG N-glycans played a critical role in anti-inflammatory process^[47] and in this way contributed to protect people from aging and age-related diseases,^[48] such as inflammaging,^[41,49] but the physiopathological mechanisms were still controversial.^[40]

Besides the level of galactosylation, the sialylation levels were also suggested to be an essential role in the anti-inflammatory activities of IgG antibodies^[44,50] and therefore might be involved in the aging process. Disialylated biantennary glycans were reported to increase with age in children, whereas monosialylated biantennary glycans showed no statistical significant association.^[46] For the adults in our study, however, both monosialylated and bisialylated biantennary glycans were decreased with age in both males and females. Like the galactosylation, the change of sialylation level was more dramatic in females than that in males. Those phenomena indicated the different changing patterns between children and adults, and also between males and females.

To our knowledge, this is the first attempt to build a predictive model of chronological age using the information from both IgG glycans and clinical parameters in a Han Chinese population. Here, we first built 3 models (GlyAge-Pooled, GlyAge-Male, and GlyAge-Female models) and introduced 3 Glycan indexes (GlyAge-Pooled index, GlyAge-Male index, and GlyAge-Female index) accordingly to predict chronological age using information only from IgG glycans based on pooled, male, and female samples, respectively. These indexes could explain 23.2% to 45.4% of variation in chronological age with the relationship between age and predicted age of 0.40 to 0.65 in the investigated samples. Compared to the reported single age biomarkers, such as telomere length which based on the literature data can explain 15% to 25% of variance in chronological age, the GlyAge indexes in our study that integrated the information from IgG N-glycans were shown to be more closely related to chronological age.

After adjustment of chronological age, a strong correlation between the 3 GlyAge indexes and the clinical traits that related to biological ages was also observed (Table 4). Thus, IgG glycosylation appeared to be associated with both chronological and biological ages. Consistent with this, inclusion of clinical traits into the previous prediction models could improve the prediction especially for the GlyAge-Pooled model. By integrating the clinical traits, the proportion of explained variation in age increased from 29.4% to 36.1%. For the GlyCliAge-Female model as well, nearly half (48.2%) of the variation of age could be explained by the glycans and clinical traits (Table 5).

To test these 6 models and to determine a general model, we applied them to the pooled, male, and female samples separately. According to the association between predicted age and chronological age, the predictive ability of GlyCliAge-Pooled model was acceptable with correlation coefficient of 0.59 in pooled samples, 0.51 in males, and 0.61 in females, which is higher than those in the rest of models. This indicated that although both glycans and clinical traits were different between

sexes, the combined index could still be applied in all the investigated participants, which would benefit its practicability in community-based population study.

In addition to the 6 models we built, we also tried to validate, in this study population, 2 established sex-specific models for age prediction, which were built based on European population study.^[30] The correlation coefficient between the predicted and the chronological age was 0.41 (0.43) when we applied the model for males (females) in our Chinese study population. Both of these correlation coefficients were lower than those in the models built in our study. In addition to the sex difference, those observations suggested that the ethnic difference should also be considered when applying these age predictive models.

Models that can indicate biological age are of significant interest for prevention, diagnosis, and monitoring (and/or the treatment) of aging and age-related diseases. Changes of the inflammatory states have been proved to be involved in the aging process and IgG glycosylation plays an essential role in the inflammatory cascade.^[1,20,41] Therefore, the inclusion of IgG glycan information becomes the most interesting consideration in building an age predictive model. The limitation of this study is that models we introduced are built based only on the data we collected, so studies with larger sample size and different ethnic groups are needed to validate and improve the models. Also, the current correlation between N-glycans and aging needs to be further validated before its clinical application.

5. Conclusions

Our study showed that most of IgG glycans were significantly related with chronological age in Han Chinese population and we illustrated extensive patterns of the changes in IgG glycans with age. The age predictive models consisted of 12 glycan structures (4 for GlyAge-Pooled model, 6 for GlyAge-Male model, and 8 for GlyAge-Female model) and explained 23.2% to 45.4% of variance in age. The remaining variance in these glycans was associated with clinical traits related to biological age. Therefore, IgG glycosylation seems to correlate with both chronological and biological ages and thus contribute to aging process. It was further supported by including the clinical traits such as SBP, LDL, and urea in the prediction models, which significantly increased the prediction and cumulatively explained 48.2% of variation in chronological age in females. Models that can indicate biological age are of significant interest for prevention, diagnosis, and monitoring (and/or the treatment) of aging and age-related diseases.

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